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"BIOPRODUCTION OF INDOLE-3-ACETIC ACID IN PRESENCE OF VITAMIN B6 AND B12 BY BACILLUS SUBTILIS AND BACILLUS AMYLOLIQUEFACIENS"

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ABSTRACT

The present study is aimed to investigate the effect of two water soluble vitamins namely Vitamin B6 and B12 on Indole-3-acetic acid (IAA) production by Bacillus amyloliquefaciens and Bacillus subtilis under in vitro condition, in L-tryptophan independent condition. IAA production was checked in presence of Vitamin B6 and B12 at a concentration of 0, 50 and 100 µg/ml in JNFb$^-$ minimal medium additionally containing 2.5mM ammonium chloride. Quantitative estimation of IAA production was carried out after 96h at 37^{0}C. Vitamin B6 followed by Vitamin B12 acts as the most preferred source for IAA production. Among the five strains, Bacillus Subtilis strain name WR-W2 was the most efficient strain in terms of its capability to produce IAA in presence of vit.B6. However, the level of IAA was very low in presence of vitamins as compared to only tryptophan added medium. Taken together, the result shows that vit.B6 and vit.B12 significantly stimulate IAA production by Bacillus sp. Altogether; the strains might be implicated toward sustainable agriculture.

KEYWORDS: IAA, Bacillus, Pyridoxine, Cyanocobalamin, B6, B12

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Original Article

INTRODUCTION

Bacillus sp. is a major component of the soil microflora beneficial to plant growth activities (Mishra and Kumar 2015a). Soil bacteria inhabit a complex and highly variable environment. *Bacillus sp*, a ubiquitously distributed, mostly dominated soil organism, is metabolically versatile and displays adaptation to changing environmental conditions (Kunst 1997). *Bacillus* sp. possess important physiological characteristics including production of a multilayered cell wall structure, formation of stress-resistant endospores and secretion of peptide antibiotics, peptide signal molecules, and extracellular enzymes, for the survival (Gardener, 2004). Therefore due to significant abilities for survival these bacteria inhabit diverse niches in agro-ecosystems. One of the mechanisms of phytostimulation and plant growth promotion involves biosynthesis of IAA by root colonizing bacteria. Soils usually contain several vitamins originating from different sources such as soil microbial activities (Kozlova, 1970), Rhizodeposition of sloughed off cells, plant secreted mucilages and soluble exudates resulted by the cell lysis are the most common sources making soil rhizosphere nutrient rich, as it contains carbohydrate monomers, amino acids, organic acids, phytosiderophores, flavonoides, plant hormones and vitamins (jonese*t al*., 2004). Vitamins are essential dietary compounds for many organisms. Mostly water-soluble vitamins function as cofactors in enzymatic reactions. *Bacillus* sp. is a major component of soil microfora and has been

reported to be phytostimulant to various crop plants plant. Literature survey reveals biosynthesis of IAA by *Bacillus subtilis* as well as *Bacillus amyliquefaciens* has been well documented (Chen et al., 2005, Mishra and Kumar 2015; Lochhead and Thexton 1951; Lochhead and Burton 1957; David et al., 2004). The present study aimed to investigate the effect of vitamin B6 and B12 on the IAA production by *B. subtilis,* and *B. amyloliquefaciens* under *in vitro* conditions.

MATERIALS AND METHODS

Isolates, Culture Media and Incubation Condition

To isolate most efficient IAA producing PGPR, soil samples were collected from rice, wheat and maize agricultural crop field and the procedure followed as per our previous publication described in Mishra and Kumar (2015a). with slight modification from one kg of rhizospheric soil. Isolation of bacteria was performed following the methods of Barraquio *et al.,* (2000). The isolates were initially grown and maintained onto Luria Bertani (L.B.) medium (Sambrook and Russell, 2001). Pure culture of the rhizospheric bacterial isolates were performed by three to four times streaking and repeated sub-culture onto modified Johanna Nitrogen fixing bacteria (JNFb) solid medium containing Malic acid 5.0g/l, K2HPO4 0.60 g/l, KH2PO4 1.80g/l, NaCl 0.10g/l, MgSO47H2O 0.20g/l, Na2MoO4.2H2O 0.002g/l, KOH 4.5g/l, CaCl2.2H2O 0.20Gg/l, Fe.- EDTA (1.4%) 4.0ml and NH4Cl, 2.5mM at pH 5.8 (Dobereiner *et al.,* 1995). Five most efficient IAA producing rhizospheric bacteria were selected namely MR-SP, MR-Z1, MR-AI, WR-W2 and RR-R2. IAA test was performed in JNFb liquid medium exogenously supplemented with L-tryptophan (100µg/ml).

Quantitative Estimation of IAA Production in Presence of Vitamins

To check IAA production by the strains in JNFb medium additionally supplemented with 2.5 mM NH_4Cl along with 0, 50 and 100 µg/ml of vitamin B6 and B12, separately, in duplicate set for all the culture tubes of five strains. $1x10^6$ concentrations of inoculums was transferred into 10ml of freshly prepared medium, incubated in 50 ml Borosil glass tubes with three replicates at 37^0C on a rotary shaker for 96h. The optimum time for maximum IAA production growth was measured turbidimetrically by an EEL (Evans Electroselenium Ltd, Engaland) calorimeter at 530 nm following calorimetric estimation of IAA (Gordon and Weber 1952).

DNA Sequencing

The 16S rRNA gene sequences were submitted to the National Center for Biotechnology Information database (NCBI) on http://www.ncbi.nlm.nih.gov. Nucleotide sequence accession numbers of 16S rRNA gene sequences of the five strains are MR-AI (1460 bp), identified as *Bacillus amyloliquefaciens* [FJ222551]. Strain WR-W2, (1473 bp) identified as *B. subtilis* [FJ222553]; Strain MR-Z1 (1459 bp) identified as *B. subtilis* [FJ269243]; Strain RR-R2 (393 bp) identified as *B. subtilis* [EU327502] and Strain MR-SP (384 bp) identified as *B. subtilis* [EU327504].

RESULTS AND DISCUSSIONS

Phyostimulatory potential of rhizospheric bacteria has been well documented (Ahemad and Kibret 2013)**.** However, hardly any study has been reported to date regarding effect of vitamin B6 and B12 on the IAA production in the presently studied five rhizobacterial strains. Diverse levels of IAA production were observed as represented in figure: 1. and figure.2. shows vitamin B6 proved to be more suitable than vitamin B12 source for IAA production activity. The highest level of IAA was observed by isolate WR-W2 in presence of vitamin B6 (6.6µg/ml) and B12 (2.5µg/ml). More than two fold increase in IAA production level was observed in presence of B6 as compared to B12 at concentration of 50µg/ml and 100µg/ml separately. Strain *Bacillus Subtilis* WR-W2 was observed as the most efficient IAA producer

followed by *Bacillus subtilis* strain *R*R-R2 in presence of vitamins B6 and B12. Effect of Vitamin B6 and B12 on IAA production in *Bacillus sp*. has not been well documented. Below the concentration of 50µg/ml IAA production was also observed but the level was too low to be represented here. However, effect of L-tryptophan on IAA production has been well documented (Mishra and Kumar 2015). Moreover, the level of IAA production was twofold higher in presence of only L-tryptophan added JNFb medium as compared to only vit B6/ B12 added JNFb medium under similar condition.On the other hand IAA production was also checked in L-tryptophan added medium along with B6 and B12, in separate culture tubes under the same conditions. The level of IAA was observed little bit higher as compared to only B6 and B12 added medium but lower than the only L-trytptophan added medium under similar condition by all the five strains. Biosynthesis of IAA by rhizospheric bacteria has been revealed in several reports (Mishra and Kumar 2012). Effect of water soluble vitamins on the IAA production has been well studied in *Azospirillum brasilense* (Zakharova et al., 2000). Vitamins may also play a role in the regulation of IAA synthesis in *Bacillus* sp. (Ahemad and Kibret 2013)**.**The active intracellular form of vitamin B6, pyridoxal 5-phosphate (PLP), has multiple roles as cofactor of enzymes. Soils can be regarded as one of the richest natural sources of B12. Altogether, significant amount of IAA production was observed in presence of vit. B6 and B12 by the five strains.

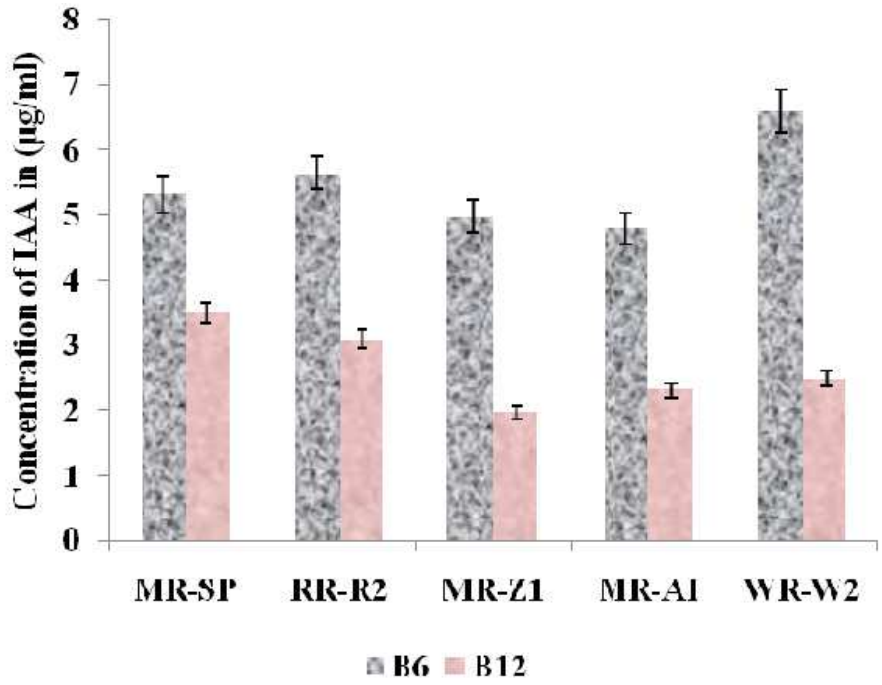

Figure 1: Effect of Vitamins B6 and B12 at Concentration of 50µg/Ml on the IAA Production.

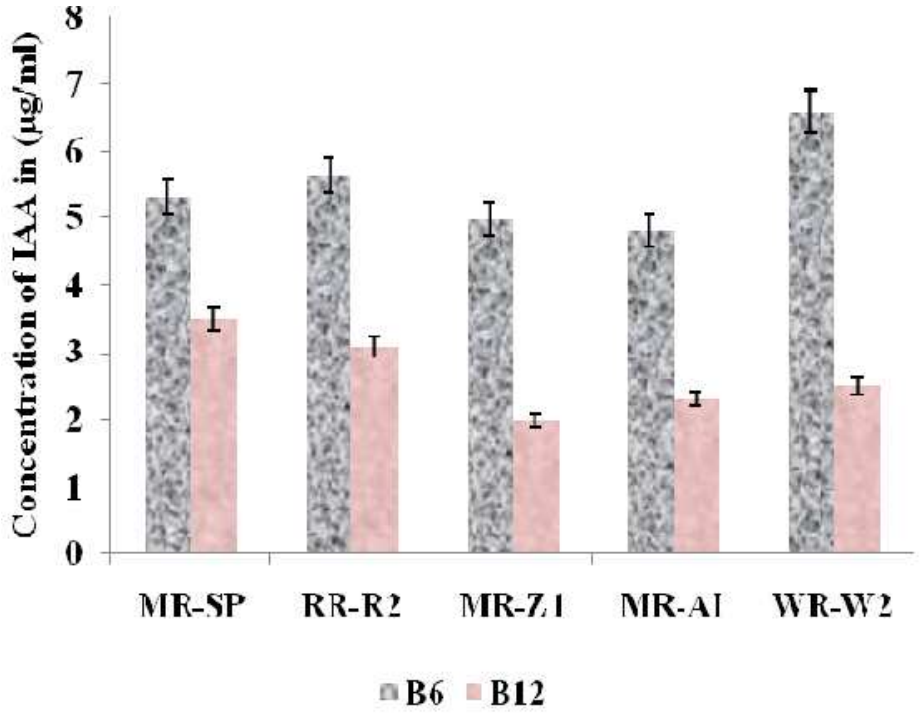

Figure 2: Effect of Vitamins B6 and B12 at Concentration of 100µg/Ml

CONCLUSIONS

To our knowledge, this is the first report of demonstrating release of IAA in presence of vit B6 and B12 by *Bacillus* spp. Which have sustainable agricultural significant importance and could be used as model organism for phytostimulation. The present study need further research so that the strains could be directly applied to crop fields in different formulations for sustainable agriculture.

REFERENCES

1. *Ahemad M, Kibret K (2013). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Univ. Sci. 26:1-20.*
2. *Barraquio WL, Segubre EM, Gonzalez MS, Verma SC, James EK, Ladha JK, Tripathi AK (2000). Diazotrophic enterobacteria: what is their role in rhizosphere of rice? In: J.K. Ladha and P.M. Reddy, Editors. The Quest for Nitrogen Fixation in Rice, Los Banos, Philippines. pp. 93-118.*
3. *Chen XH, Koumoutsi A, Scholz R, Schneider K, Vater J, Sussmuth R, Piel J, Borriss R. (2009) Genome analysis of Bacillus amyloliquefaciens FZB42 reveals its potential for biocontrol of plant pathogens. J Bacteriol 140, 27–37.*
4. *David LJ, A Hodge, Yakov K (2004). Tansley review Plant and mycorrhizal regulation of rhizodeposition. New Phytologist. 163: 459–480.*
5. *Dobereiner J. (1995). Isolation and identification of aerobic N2-fixing bacteria from soil and plants. In: Methods in Applied Soil Microbiology and Biochemistry, (Alef and P. Nannipieri Eds.). Academic Press, London, U.K. pp. 134-141.*
6. *Lochhead AG, Thexton RH (1951) Vitamin B_{12} as a growth factor for soil bacteria. Nature 167, 1034.*
7. *Lochhead AG, Burton MO (1957) Qualitative studies of soil microorganisms. Specific vitamin requirements of the predominant bacteria flora. Can. J. Microbiol. 3, 35–42.*

8. *Gordon SA, Weber RP (1951) Colorimeteric estimation of indole acetic acid. Plant Physiol 26: 192-195.*

9. *Gardener BBM (2004) Ecology of Bacillus and Paenibacillus spp. In Agricultural Systems. (Ed.), Symposium: The Nature and Application of Biocontrol Microbes: Bacillus spp. Phytopathology.pp. 1252-1258.*

10. *Kozlova IA, (1970) Biosynthesis of group B vitamins by soil mycobacteria. Chem. Abstr. 73, 53198.*

11. *Kunst F, N Ogasawara, I Moszer, AM Albertini, G Alloni, V Azevedo et al. (1997). The complete genome sequence of the grampositive bacterium Bacillus subtilis. Nature 390:249–256.*

12. *Mishra VK, Kumar A (2015). Biosynthesis of indole-3-acetic acid by plant growth promoting rhizobacteria, Klebsiella pneumonia, Bacillus amyloliquefaciens and Bacillus subtilis.African Journal of Microbiology Research. 9:16:1139-1149.*

13. *Mishra VK, Kumar A, (2015a) In vitro assessment of plant growth promoting potential of Klebsiella pneumoniae, Bacillus amyloliquefaciens and Bacillus sp. International Journal of Agricultural Science and Research. Vol. 5, Issue 3, Jun 2015, 69-80.*

14. *Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring harbor, New York, USA.*

15. *Zakharova EA, Iosipenko AD, Ignatov VV (2000). Effect of water-soluble vitamins on the production of indole-3-acetic acid by Azospirillum brasilense. Microbiol. Res. 155:209-214.*